EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Calibration and Maintenance of pH Meters

SOP Number: EQ-01-04

Date Revised: 08-03-05

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Controlled Copy No.: _____

Withdrawn By: _____Date: ___/___/___

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION:

1.1 This protocol describes the method for the operation, two point calibration, and maintenance of the Corning pH meter 430 and the Corning pH meter 530.

2.0 DEFINITIONS:

2.1 A two-point calibration involves calibrating the pH meter with two different buffers of known pH and then checking the pH meter against a pH 7.00 standard buffer to confirm the calibration.

2.2 Ceramic Junction = Holds the fill solution in the electrode. The junction is designed to allow the fill solution to leak out of the tip of the electrode at a slow controlled rate.

2.3 Fill Hole Plug = Small circular hole on the side of the electrode that is covered by a rubber stopper when not in use. The fill whole plug is removed during calibration and maintenance of the electrode or when fill solution (KCL) is being added to the inside of the electrode.

2.4 Fill Solution = Acts as an electrical conductor between the reference inside the electrode and the solution under test.

2.5 Wetting Cap = The cap that covers and protects the tip of the electrode. The wetting cap should stay on the tip of the electrode during periods of non use.

3.0 HEALTH AND SAFETY:

3.1 Personal protective equipment including safety glasses and lab coats should be used.

4.0 CAUTIONS:

4.1 Do not allow fill solution to run dry. Add fill solution whenever the level falls more than 25 mm below the fill hole. Replace solution at least once a month.

4.2 Always remove the wetting cap and the fill hole plug during calibration and measurements. Replace the fill hole plug in between use.

4.3 Do not leave the electrode in organic solvents as the tip and body may be

damaged.

4.4 Discard used buffer solutions daily. Do not reuse.

5.0 INTERFERENCES:

5.1 The pH meter electrode should be rinsed thoroughly with de-ionized water and blotted dry with Kimwipes® (wiping can produce a static charge) before proceeding to the next solution.

5.2 The electrodes can be stored for up to one week in 25 mm of pH 7.0 or pH 4.0 buffer. Never store the electrode in distilled water.

5.3 For longer storage periods, remove the bulb protector, fill the wetting cap with KCl solution and push the wetting cap onto the tip of the electrode.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP as well as the operation hints and maintenance sections of the pH meter instruction manual.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Corning pH Meter #1: Model 430 (Serial No. 005166)

7.2 Corning pH Meter #3: Model 530 (Serial No. 301887)

7.3 Corning pH Meter #4: Model 530 (serial No. 304953)

7.4 Electrodes:

7.4.1 Six '3 in1' Combo w/RJ (Epoxy body) (Corning Catalog No. 76436). Electrode performance specifications (Manufacturer's claim): the pH range 0.0 -14.0 and the temperature range 0 - 100°C. The electrodes are identified as #1, 2, 5, 6, 7, and 8. These electrodes are used for pH measurement of liquids.

7.4.2 Two Flat-Surface (Epoxy Body) with Replaceable Junction Electrode (Corning Catalog No. 476286). Electrode performance

specifications (Manufacturer's claim): the pH range is 0.0-14.0 and the temperature range is 0-100°C. The electrodes are identified as # 3 and # 4. These electrodes are used for pH measurement of solid media.

7.4.3 One Semi-Micro Glass Body with Refillable Junction Combo Electrode (Corning Catalog No. 476156). Electrode performance specifications (Manufacturer's claim): the pH range is 0.0-14.0 and the temperature range is 0-100°C. Electrode is identified as #9. The electrode is used for measuring pH of small aliquots (≤ 1 mL) of media.

7.5 Calibration Buffers:

pH 4.0
pH 7.0
pH 10.0

7.6 Filling solution: 3M KCl

7.7 Storage Solution: 3M KCl for capping OR pH 4.0 or pH 7.0 buffer for buffer immersion.

7.8 Pepsin Deproteinizing solution

7.9 Ceramic replacement junction

7.10 1N Sodium Hydroxide Solution (NaOH)

7.11 1N Hydrochloric acid (HCl)

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 As described in Procedure and Analysis section.

9.0 SAMPLE HANDLING AND STORAGE:

9.1 The pH of media and reagents are to be taken when the media and reagents are at room temperature (25 ± 2ºC) unless otherwise specified on the Media/Reagent preparation sheet. For agar based media, the pH is to be taken on a solidified

sample (see 10.5).

9.2 The buffers (see 7.5) and reagents (1N HCl and 1N NaOH) are stored adjacent to the pH meter at room temperature in a secondary containment unit.

10.0 PROCEDURE AND ANALYSIS:

10.1 The pH meter is calibrated at least once on the day of use as outlined in section 10.3. The standard buffers used are pH 4.0, pH 7.0, and pH 10.0. A small amount is dispensed into a smaller container for calibration. Per manufacturers recommendations the standard pH buffers should be used for calibration only one time and disposed after calibration has been finalized.

10.2 Selection of Buffers for 2-Point Calibration:

10.2.1 If the final pH of the reagent or medium falls between a pH of 7.0 and 10.0, use the pH 7.0 and 10.0 calibration buffer solutions for the two point calibration.

10.2.2 If the final pH of the reagent or medium falls between a pH of 4.0 and 7.0, use the pH 4.0 and 7.0 calibration buffer solutions for the two point calibration.

10.2.3 The expiration dates of the buffers are recorded on the upper part of the pH Meter Calibration Record Form (see 16.0). Record the date the buffers are changed under 'Notes' on the form.

10.3 Two Point Calibration

10.3.1 Model 430

10.3.1.1 Check the level of the 3M potassium chloride (KCl) electrode filling solution to ensure that it is within 25 mm of the filling hole. Bring to level if needed. In the Corning pH Meter # 1 Calibration Log Book, record under "Notes" any adjustments made with the filling solution.

10.3.1.2 To calibrate, rinse the probe tip with de-ionized water and blot dry with Kimwipes®, then place the tip of the electrode into the pH 7.0 buffer solution and press "Cal."

10.3.1.3 The pH meter will automatically read the endpoint when the reading is stable. The appropriate buffer symbol will appear on the display. Record the pH value on the pH Meter Calibration Check Record Form (see 16.0).

10.3.1.4 Rinse the tip of the electrode with de-ionized water and blot dry with Kimwipes®.

10.3.1.5 Place the tip of the electrode in the second calibration buffer (either calibration buffer pH 4.0 or calibration buffer pH 10.0) and press "Cal".

10.3.1.6 The pH meter will automatically read the endpoint when the reading is stable and the appropriate buffer symbol will appear on the display.

10.3.1.7 The pH meter will also briefly display the electrode slope value. Record the pH value on the pH Meter Calibration Check Record Form (see 16.0). If the slope is less than 95% or greater than 105%, refer to the troubleshooting section of the Corning pH meter 430 instruction manual (see ref. 15.3).

10.3.1.8 Rinse the electrode with de-ionized water and blot dry with Kimwipes®. Confirm the calibration by reading the pH of a standard pH 7.0 buffer a second time. Record the pH value in the pH Meter Calibration Check Record Form (see 16.0). Press "Mode" one time to switch to temperature mode. Record the temperature value in the pH Meter Calibration Check Record Form (see 16.0). Press "Mode" two more times to return to pH mode.

10.3.2 Model 530

10.3.2.1 Check the level of the 3M potassium chloride (KCl) electrode filling solution to ensure that it is within 25 mm of the filling hole. Bring to level if needed. In the Corning pH Meter # 3 and # 4 Calibration Log Book, record under "Notes" any adjustments made with the filling solution.

10.3.2.2 Verify that the pH meter is set at "Auto endpoint" as indicated by a letter "A" in the upper left hand corner of the display. If the letter "A" is not indicated, press the "Auto" button until it is displayed. After the endpoint is reached, brackets will appear around the "A" (e.g [A]). The temperature and slope will appear at the bottom of the display. When selecting the buffer for calibration, the selected buffer pH will also appear at the bottom of the display, but once the calibration begins, the pH of the buffer will disappear.

10.3.2.3 To calibrate, rinse the probe tip with de-ionized water and blot dry with Kimwipes®, then place the tip of the electrode into the pH 7.0 buffer solution and press "Cal" one time so that "pH 7" and "Cal 1" appear.

10.3.2.4 The pH meter will automatically read the endpoint when the reading is stable. The appropriate buffer symbol will appear on the display. Record the pH value on the pH Meter Calibration Check Record Form (see 16.0).

10.3.2.5 Rinse the tip of the electrode with de-ionized water and blot dry with Kimwipes®.

10.3.2.6 Place the tip of the electrode in the second calibration buffer. If pH buffer 4.0 is used, press "Cal" one time so that "pH 4" and "Cal 3" appear. If pH buffer 10.0 is used, press "Cal" two times so that "pH 10" and "Cal 3" appear.

10.3.2.7 The pH meter will automatically read the endpoint when the reading is stable and the appropriate buffer symbol will appear on the display. The display will show the electrode slope value. Record the pH value and slope on the pH Meter Calibration Check Record Form (see 16.0). If the slope is less than 95% or greater than 105%, refer to the troubleshooting section of the Corning pH meter 430 instruction manual (see ref. 15.3).

10.3.2.8 Rinse the electrode with de-ionized water and blot dry with

Kimwipes®. Confirm the calibration by reading the pH of a standard pH 7.0 buffer a second time. Record the pH value and temperature in the pH Meter Calibration Check Record Form (see 16.0).

10.4 Reading the pH of a liquid sample:

10.4.1 The pH of the liquid sample should be taken at room temperature unless otherwise specified on the Media/Reagent preparation sheet.

10.4.2 Place the tip of the sensed and dried electrode in the medium or reagent sample and press "read" to start the measurement. The decimal point will flash while the electrode is reading.

10.4.3 Record the temperature of the sample.

10.4.3.1 For pH model 430, press "Mode" one time to switch to temperature mode. The display will show the temperature in °C. Record on the Media/Reagent preparation sheet the temperature at which the solution's pH was measured. Press "Mode" two more times to return to pH mode.

10.4.3.2 For pH model 530, the temperature will display at the same time as the pH. The display will show the temperature in °C. Record on the Media/Reagent preparation sheet the temperature at which the solution's pH was measured.

10.4.4 When the pH reading is stable, record the initial pH of the solution on the Media/Reagent preparation sheet (see SOP MB-10, Media and Reagents Used in Efficacy Testing).

10.4.5 If the pH of the liquid falls outside of the desired range as specified on the media/reagent preparation sheet, adjust the pH of the tempered, liquid media using the specified acid and base solutions and record the amount used on the Media/Reagent preparation sheet.

10.4.6 Record the adjusted pH of the solution on the Media/Reagent

preparation sheet.

10.5 Reading the pH of a sample containing agar:

10.5.1 The pH of the agar sample should be taken as a solid. From the total volume of the media prepared, place an aliquot (approximately 5-10 mL) into a petri dish or small weigh boat and let solidify; keep the remainder of the media between 45-50°C (in case the media needs to be adjusted). After the media is solidified, use the Flat-Surface (Epoxy Body) with Replaceable Junction Electrode to measure the pH of the media (see ref. 15.4).

10.5.2 Place the electrode on the surface of the solidified media and press "Read" to start the measurement. The decimal point will be flashing whenever the electrode is reading.

10.5.3 When the pH reading is stable, record the pH of the media on the Media/Reagent preparation sheet (see SOP MB-10, Media and Reagents Used in Efficacy Testing).

10.5.4 If the pH of the agar falls outside of the desired range (see 10.5.1 above) as specified on the media/reagent preparation sheet, adjust the pH of the tempered, liquid agar (prior to autoclaving) using the specified acid and base solutions. Discard any media that cannot be adjusted.

10.5.5 Repeat steps 10.5.1 through steps 10.5.4 as needed. Record the adjusted pH of the solution on the Media/Reagent preparation sheet.

10.5.6 The pH of some media (e.g. TSA) is tested after autoclaving. Follow the instructions on the media/reagent preparation sheet. Place an aliquot (approximately 5-10 mL) of the medium into a petri dish or small weigh boat, let solidify; then take the pH of the solidified agar. Record this reading on the Media/Reagent preparation sheet.

10.6 Cleaning and Maintenance of the electrode:

10.6.1 Cleaning should be done when the slope falls below 95% or is

greater than 105%.

10.6.2 Test the flow of the junction by letting the electrode hang in the air for 1-2 hrs. A proper flowing junction will have KCl salt crystals forming on it. If none appear, review the following suggestions for cleaning.

10.6.2.1 For protein contamination: Soak the tip of electrode in deproteinizing solution for 1-2 hrs. Rinse the electrode with de-ionized water and soak in pH 7.0 buffer until stable.

10.6.2.2 For oil contamination: Carefully clean the tip of the electrode using a cotton swab soaked with alcohol or acetone. Rinse the electrode with de-ionized water and soak in pH 7.0 buffer until stable.

10.6.3 If cleaning does not improve junction flow, the junction will be replaced. To replace, simply pull out the clogged junction, rinse the electrode barrel with de-ionized water, and replace with a clean junction. Then refill electrode with appropriate solution up to the fill hole plug (see ref. 15.2).

10.6.4 After cleaning and maintenance activities, check the calibration of the pH meter and record on the pH Meter Calibration Form.

10.6.5 Record cleaning and maintenance activities under notes on the pH Meter Calibration Record Form.

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Calibration data will be recorded promptly, legibly and in indelible ink on the Corning pH Meter Calibration Record form(s) (see 16.1). Completed forms are archived in notebooks kept in secured file cabinets in the file room D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The pH meter is cleaned when the slope falls below 95% or is greater than 105%.

13.2 For quality control purposes, the required information is documented on the appropriate forms (see 16.1).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 The pH meter in order to pass calibration has to display ≥ 95% slope, if the pH meter does not display a slope between 95% and 105% the first corrective action is to change the standard pH buffers and recalibrate. The second step is to change the KCl solution or bring up to volume, then re-calibrate. The final corrective action is to clean the electrode with deproteinizing solution (follow manufacturers instructions). Any corrective action taken in-house is recorded under 'Notes' on the Corning pH Meter Calibration Record Form (see 16.1). If the pH meter is not functioning as required, consult the technical manual to determine the problem and perform corrective action. Notify media prep staff, and if the problem cannot be determined or corrected, call for service.

15.0 REFERENCES:

15.1 Corning "Electrodes" Information sheet for pH combination 3-in-1 electrodes.

15.2 Corning- Labware & Equipment- Electrodes:" How to Maintain The Premium Performance of Your Corning Electrodes"

15.3 Instruction manual for Corning pH meter 430.

15.4 Instruction manual for Corning pH meter 530.

15.5 Corning "Electrodes" Information pamphlet for flat surface electrodes.

16.0 FORMS AND DATA SHEETS:

16.1 Corning pH Meter Calibration Record Form.

Corning pH Meter Calibration Record pH Meter #___
OPP Microbiology Laboratory

Standard Buffer	Control Number
pH Buffer 4.0	
pH Buffer 7.0	
pH Buffer 10.0	

Date	Initials	Temperature of Calibration	Slope (%)	Electrode No.	Buffers Used for Calibration			Re-check pH 7.0
					pH 4.0	pH 7.0	pH 10.0	
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